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2017

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Palonen , P , Pinomaa , A & Tommila , T K 2017 , ' The influence of high tunnel on yield and berry quality in three floricane raspberry cultivars ' , Scientia Horticulturae , vol. 214 , pp. 180-186 . <https://doi.org/10.1016/j.scienta.2016.11.049>

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<http://hdl.handle.net/10138/308521>

<https://doi.org/10.1016/j.scienta.2016.11.049>

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1 The influence of high tunnel on yield and berry quality in three florican raspberry cultivars

2

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6

7 Abstract

8 Growing raspberries in polyethylene tunnels is becoming more and more common. We wanted to examine  
9 the effect of high tunnel growing conditions on yield and berry quality in three florican raspberry cultivars,  
10 'Glen Ample', 'Glen Dee', and 'Maurin Makea', under Northern high-latitude conditions. Compared to the  
11 open field, fruit yield per cane was doubled in the tunnel. Fruit bioactive properties, including phenolic  
12 compounds and antioxidant activity, were not affected by the tunnel growing conditions. Of the cultivars  
13 investigated, 'Glen Dee' fruit had the lowest concentration of total phenolics. In the open field, the total  
14 phenolics content in 'Glen Ample' berries was 48% higher than 'Glen Dee'. Berries grown in the open field  
15 had higher contents of soluble solids (°Brix) and higher titratable acidity than those grown in the tunnel.  
16 Additionally, 'Glen Ample' and 'Maurin Makea' berries were sweeter than 'Glen Dee' berries. In conclusion,  
17 raspberry production in polyethylene tunnels may provide major benefits through increased fruit yield.  
18 While fruit bioactive properties were not affected, sensory taste may be different, however, as berry  
19 sweetness and acidity were decreased in the high tunnel.

20

21 Keywords: acidity, antioxidant activity, phenolics, *Rubus idaeus*, soluble solids

22

23 1. Introduction

24 Raspberry (*Rubus idaeus*) production in the world has nearly doubled over the past 20 years (FAOSTAT,  
25 2016). Growing public awareness of the putative health benefits of berries and greater interest in healthy  
26 diets has increased the demand of the fruit. Raspberries are commonly sold in the fresh market, and long  
27 shelf life is an important quality parameter. Because of the superior fruit quality and shelf life when  
28 produced in a protected environment, more and more raspberries are grown in polyethylene tunnels. In  
29 some countries, in fact, this is a requirement set by the supermarkets. Polyethylene tunnels have also been  
30 reported to increase yields and extend the season (Hanson et al., 2011; Fernandez and Perkins-Veazie,  
31 2013; Xu et al., 2014).

32 Many environmental parameters in the tunnel are different from the open field, including light intensity  
33 and quality, temperature, humidity, wind, as well as pest and disease pressures. In high tunnels, long cane  
34 plants are commonly used and grown in substrate. Little information, however, is available on the influence  
35 of these altered growing conditions on internal berry quality including taste, and nutritional and health-  
36 related compounds, especially in Northern high-latitude conditions.

37 The perceived sweetness in raspberry fruit comes from sugars, 40-50% being fructose, 30-40% glucose, and  
38 10-20% sucrose (Wang et al., 2009). Acidity is caused by the high concentration of organic acids, citric acid  
39 being the most important. Usually warm and dry weather increases sugar content and decreases acidity in  
40 raspberry fruit (Jennings, 1988; Malowicki et al., 2008). However, lower sugar content was observed in the  
41 primocane-type raspberry 'Polka' fruit when grown in high tunnels compared to an open field environment  
42 (Król-Dyrek and Siwek, 2015). This study indicates parameters other than the temperature difference  
43 between the tunnel and open field influenced this quality trait.

44 *Rubus* berries are a rich source of antioxidants and other bioactive compounds. The most important  
45 antioxidants in raspberry fruit are phenolic compounds and ascorbic acid (vitamin C) (Beekwilder et al.,  
46 2005). Among the ten raspberry genotypes studied by Mazur et al. (2014a), the main phenolic compounds  
47 were ellagitannins (57 %) and anthocyanins (42 %). The contents of total phenolics, flavonoids, and  
48 anthocyanins may be used to describe the antioxidant activity and thus potential health benefits of

49 raspberry fruit (Wang and Lin, 2000; Liu et al., 2002; Anttonen and Karjalainen, 2005). Antioxidant activity is  
50 strongly correlated with the total phenolic concentration in raspberries (Deighton et al., 2000; Connor et  
51 al., 2005).

52 Temperature has contrasting effects on bioactive compounds in raspberry fruit (Remberg et al., 2010).  
53 Lower temperatures increase berry size through increased moisture content, therefore decreasing  
54 concentrations of bioactive compounds when expressed on a fresh weight basis (dilution effect). Vitamin C  
55 was an exception, which increased even on a fresh weight basis at low temperatures. However, since large  
56 berry size is an important quality parameter for commercial production, Remberg et al. (2010)  
57 recommended relatively low temperatures, 12 to 18°C, during the ripening of raspberry fruit.

58 Remberg et al. (2010) also suggested that fluctuations in temperature may enhance the accumulation of  
59 bioactive compounds in raspberry fruit, especially when compared to constant temperatures. Interestingly,  
60 long day conditions during fruit growth have also been shown to increase the concentrations of vitamin C,  
61 total phenolics, organic acids, and antioxidant capacity, while reducing the sugar content in raspberry fruit  
62 (Mazur et al., 2014b). In addition to photoperiod, light spectral composition may also affect berry quality; in  
63 our previous study, ellagic acid in raspberry fruit was increased under the film absorbing far red light, while  
64 the sugar:acid ratio was slightly reduced (Palonen et al., 2011).

65 The aim of our present study was to examine how high tunnel growing conditions affect the yield and berry  
66 quality, including sugar content, acidity, and the contents of total phenolics, as well as the antioxidant  
67 capacity, in three floricane raspberry cultivars under Northern conditions.

68

## 69 2. Material and Methods

### 70 2.1. Plant material and experimental design

71 The experiment was conducted at the University of Helsinki research field in Viikki (60°13' N; 25°1' E) during  
72 growing season 2015 using floricane raspberry cultivars 'Glen Ample', 'Glen Dee', and 'Maurin Makea'. The

73 experiment was set up as an identical RCBD in a polyethylene tunnel and an adjacent open field. In each  
74 environment (tunnel versus field), plants were grown in three rows (blocks). In the tunnel environment,  
75 each row had five plants each of the three different cultivars, while in the open field, each row had six  
76 plants each of the three different cultivars. This experiment was part of a larger variety trial which included  
77 six different raspberry genotypes.

78 'Glen Ample' and 'Glen Dee' are releases by the James Hutton Institute, Scotland. 'Maurin Makea' was  
79 discovered as an open-pollinated seedling in a raspberry breeding population at the Natural Resources  
80 Institute, Finland and released in 1996. It is known for its excellent winter hardiness and good fruit flavor.

81

## 82 2.2. Growing conditions

83 The experiment was established in 2014 in sandy soil on a gentle South-facing slope. In the open field, the  
84 raised raspberry beds were covered with black woven polypropylene fabric (MyPex®). White clover  
85 (*Trifolium repens*, cv. Grassland Huia) at the rate of 10 000 seeds/m<sup>2</sup> was sown between the rows. The  
86 polyethylene tunnel (8 m × 35 m, 4 m high) was oriented South-to-North and covered with clear  
87 polyethylene (Folitec UV M 42, Folitec, Westerburg, Germany). In the tunnel, the plants were grown in 10-L  
88 pots filled with peat (OPM 630 W, Kekkila Oy, Vantaa, Finland). The tunnel floor was covered with white  
89 woven polypropylene fabric (MyPex®). The three rows were spaced 2.40 m apart in the tunnel and 2.60 m  
90 apart in the open field. Plant spacing within a row was 40 cm in the tunnel and 50 cm in the open field.

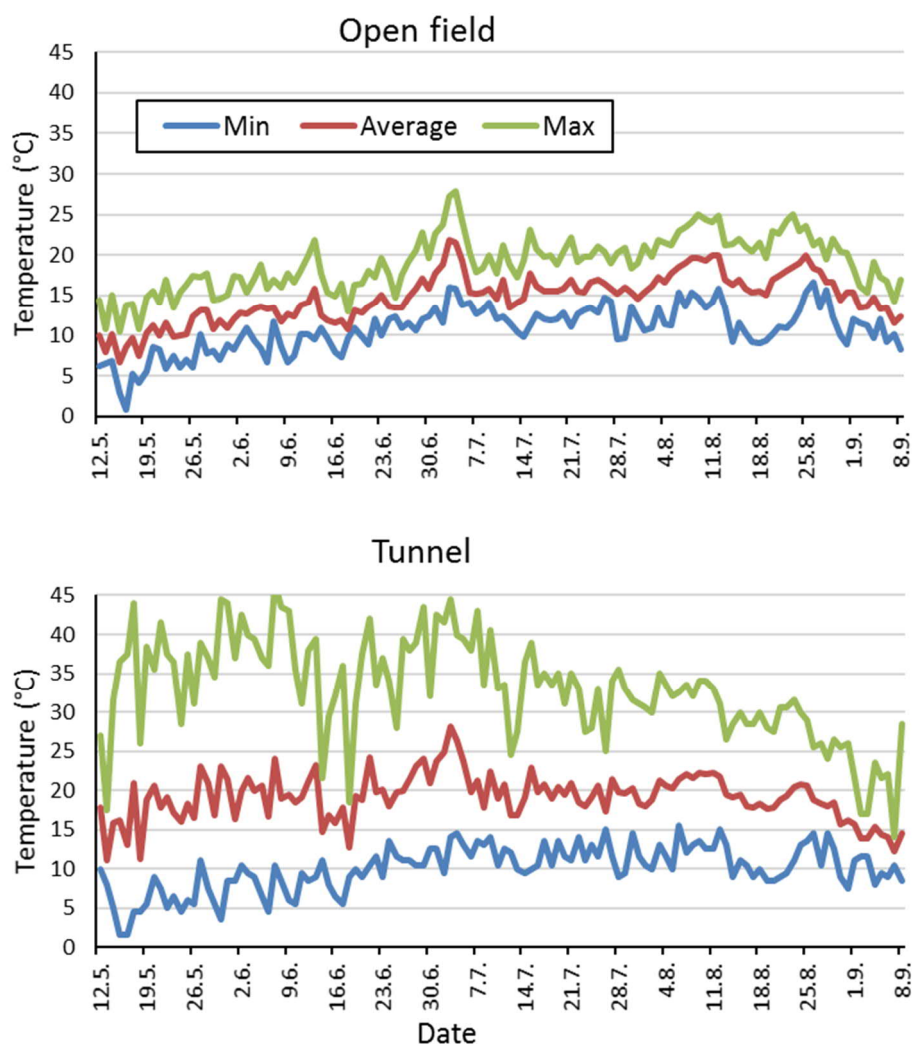
91 Tunnel plastic was removed for the winter on 30 October 2014 and replaced on 27 April 2015. The tunnel  
92 was ventilated mainly through the tunnel doors at either end. Tunnel plants were fertigated through drip  
93 irrigation three times a day with a 0.01% compound fertiliser, Taimi-Superex (NPK 19–4.4–20.2 plus  
94 microelements) (Kekkila Oy, Vantaa, Finland), from 16 May through 5 June, and with a mixture of Taimi-  
95 Superex and Turve-Superex (NPK 12–4.7–27.1 plus microelements) (0.08%) from 6 June through 19 August.  
96 Plants in the open field were fertigated through trickle irrigation system using the same fertilisers six times

97 during the growing season. One to two floricanes per plant were grown in the tunnel and one to three in  
98 the open field. New primocanes were allowed to grow freely during the experiment.

99 A bumblebee (*Bombus terrestris*) hive (Minipol, Koppert Biological Systems, Romulus, MI, USA) was placed  
100 in the tunnel to ensure pollination. No chemical control of pests or diseases was used in the experiment. In  
101 the tunnel, biological pest control included *Amblyseius cucumeris* to control thrips, *Phytoseiulus persimilis*  
102 to control spider mites, and BerryProtect tubes containing different species (*Aphidius ervi*, *A. matricariae*,  
103 *A. colemani*, *Ephedrus cerasicola*, *Praon volucre*, *Aphelinus abdominalis*) were used to control aphids (Biotus  
104 Oy, Forssa, Finland).

105 Temperature data in the open field are from the Finnish Meteorological Institute (Figure 1). Tunnel  
106 temperatures were measured in 30-minute intervals throughout the experiment. Light spectral  
107 composition in the open field and the tunnel was recorded on 15 September 2015 (Figure 2).

108



109

110 Figure 1. Daily average, minimum and maximum temperatures in the open field and high tunnel during the  
 111 growing season 2015.

112

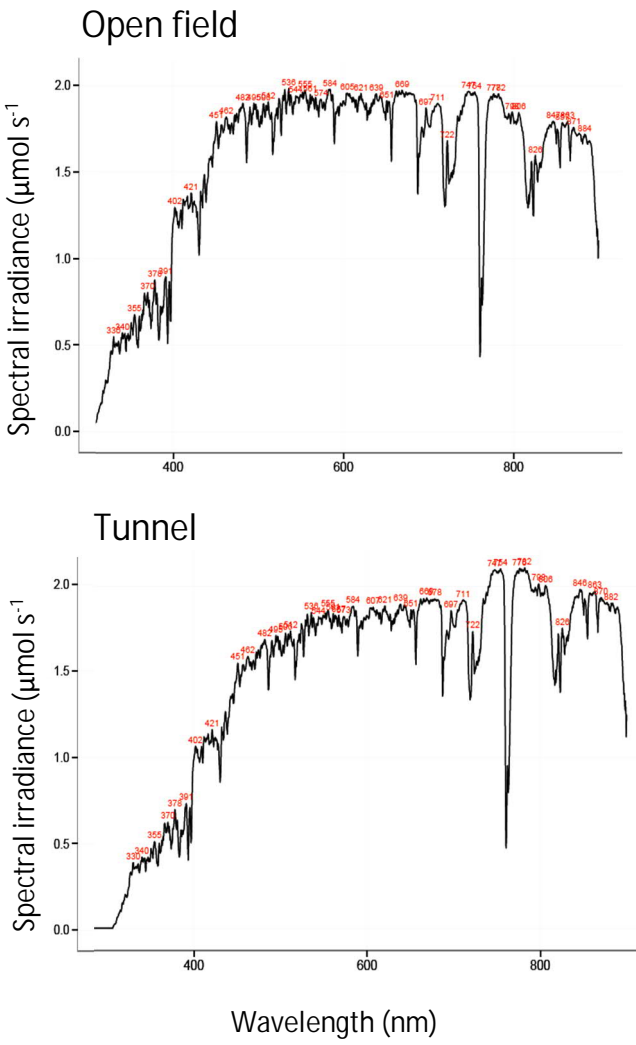


Figure 2. Spectral composition of light as a function of wavelength in the open field and high tunnel on 15 September 2015.

### 2.3. Harvesting and sample preparation

Raspberry fruits were harvested three times a week and were weighed and counted to determine total yield and the number of berries per cane. For chemical analyses of fruit quality, 150 to 200 g of berries per block were sampled and immediately frozen at -20 °C and held at -20 °C until analyses. To measure soluble solids (SS) and titratable acidity (TA), samples were taken four times during the harvest season, and three



134 times for analyses of total phenolics and antioxidant capacity. Frozen berries were thawed at room  
135 temperature (22°C) four hours before sample preparation.

136

## 137 2.4. Chemical analyses of fruit quality

### 138 2.4.1 Soluble solids, pH and titratable acidity

139 Using a hand held potato presser, 100-120 g of fruit was macerated, and then incubated in a 100 ml  
140 decanter until the upper cloudy fraction could be discarded, the clear fraction of the sample being used for  
141 the analyses. Three replicate subsamples were taken and measured for their concentration of SS (°Brix)  
142 with an analogical refractometer (Master, Atago, Japan).

143 To measure TA, a 5.0 g aliquot of clear fruit juice was added to 25 ml of ultra pure water in a decanter. The  
144 pH was then measured with a Metrohm 744 pH meter (Metrohm AG, Herisau, Switzerland) and the TA was  
145 measured with a buret and 0.1 M NaOH. The amount of NaOH needed to reach a pH of 8.1 was quantified  
146 and used to calculate the concentration (w/w) of citric acid in the sample juice; in order to neutralise 1  
147 mole of citric acid, three moles of NaOH is required.

### 148 2.4.2. Antioxidant activity and total phenolics

149 Using a blender (Waring Blendor Deluxe, Conair Corporation, NJ, USA), 100-120 g of fruit was pureed. The  
150 homogenised fruit puree (5 g) was extracted with 70% ethanol (15 ml) in a 50 ml tube at +4°C for 17 hours.  
151 According to Addai et al. (2013), recovery of antioxidant compounds using an ethanol extraction is  
152 sufficient for ferric reducing ability of plasma (FRAP) and total phenolics measurement. The samples were  
153 then centrifuged at 4600 rpm for 10 min and the pellet discarded. Lighting in the laboratory was kept dim  
154 and the tubes were wrapped in aluminum foil to prevent phenolic compounds from breaking down during  
155 the analysis. Extracts were stored at -20°C and always handled in low lighting.

156 Samples were diluted with ultra pure water 1:10 (v/v) to determine the FRAP using a modified version of  
157 the Benzie and Strain (1996) method. The FRAP reagent was prepared each day according to Benzie and

158 Strain (1996) and Deighton et al. (2000) protocols. We pipetted 1 ml of the FRAP reagent and 30  $\mu$ l of  
159 sample into a cuvette and, after incubation for 4 min at room temperature, the absorbance at 593 nm was  
160 read using a spectrophotometer (UV 1601, Shimadzu, Japan). The antioxidant activity in the sample (FRAP)  
161 was expressed as  $\mu$ mol Fe(II)-TPTZ / g FW.

162 The concentration of total phenolics (TP) was determined using the Fast Blue BB (FBBB) method (Medina,  
163 2011a). Since the optimal concentration of phenolics in the sample corresponds to a gallic acid  
164 concentration of 50-400  $\mu$ g/ml (Medina, 2011b), samples were diluted with ultra pure water 1:10 (v/v).  
165 Next, 100  $\mu$ l of 0.1% FBBB reagent and 100  $\mu$ l of 5% NaOH were added into 1 ml of sample and the mixture  
166 was incubated at room temperature for 90 mins. The absorbance at 420 nm was then read using a  
167 spectrophotometer (UV 1601, Shimadzu, Japan). The concentration of TP in the sample was expressed as  
168 mg GAE/g FW.

169

## 170 2.5. Statistical analysis

171 The experimental design was a RCBD where the three blocks were nested in a growing condition treatment.  
172 The parameters of yield and berry quality were statistically analysed by a two-way ANOVA by means of a  
173 mixed model with growing condition treatment and cultivar as the fixed factors and block (nested in  
174 growing condition) as the random factor. The SAS Mixed procedure (SAS 9.4, SAS Institute Inc.) (Littell et al.,  
175 2006) was applied to fit the model using the restricted maximum likelihood (REML) estimation method. The  
176 data from the open field and the tunnel were analysed separately using ANOVA (IBM SPSS Statistics,  
177 program 23) with cultivar as an independent factor. In cases where conditions for analysis of variance were  
178 not fulfilled, a non-parametric Kruskal-Wallis test was used. Data for berry quality measurements from  
179 different dates were pooled and the means were used in the analyses. Cultivar means were separated using  
180 Tukey's test at a significance level of  $P \leq 0.05$ .

181

### 182 3. Results

#### 183 3.1. Yield and berry size

184 Berries were harvested for 47 days in the open field and 62 days in the high tunnel. The harvest started  
185 with the cultivars 'Glen Ample' and 'Maurin Makea' on 1 July in the tunnel and 24 July in the open field (Fig.  
186 3). For 'Glen Dee', the first berries were harvested on 13 July in the tunnel and 29 July in the open field.  
187 Total yield per cane across all cultivars was, on average, 99% higher in the tunnel compared to the open  
188 field ( $P < 0.001$ ). This was due to the higher number of berries in the tunnel-grown canes ( $P < 0.001$ ), since  
189 berry size was not affected by growing condition, but was significantly affected by cultivar ( $P = 0.007$ ), with  
190 'Glen Dee' having the largest berries. Cultivars differed in total yield ( $P = 0.009$ ), as well as in their response  
191 to growing conditions due to a highly significant interaction observed between growing condition and  
192 cultivar ( $P < 0.001$ ). Pronounced cultivar differences in the total yield were observed in the open field but  
193 not in the tunnel (Table 1). 'Maurin Makea' and 'Glen Dee' produced significantly lower yields in the open  
194 field than in the tunnel ( $P < 0.001$ ), however, 'Glen Ample' was equally productive in both growing  
195 conditions (Fig. 3). For 'Maurin Makea', both berry number ( $P = 0.001$ ) and berry size ( $P < 0.001$ ) were  
196 smaller in the open field than in the tunnel. There was large variation in 'Maurin Makea' yield between the  
197 open field blocks, and a few berries suffered pest damage. Overall, berry weight declined as the harvest  
198 season progressed (Fig. 4). In the tunnel, some of the 'Glen Ample' fruit were crumbly, and, due to high  
199 variation, the differences in berry size between the cultivars were not significant in the tunnel.

200

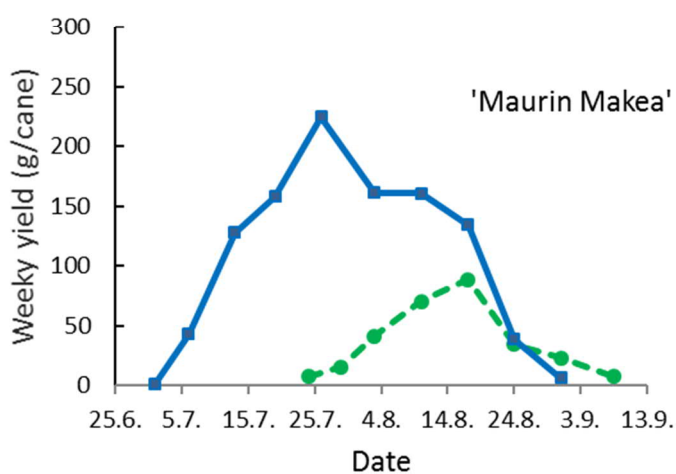
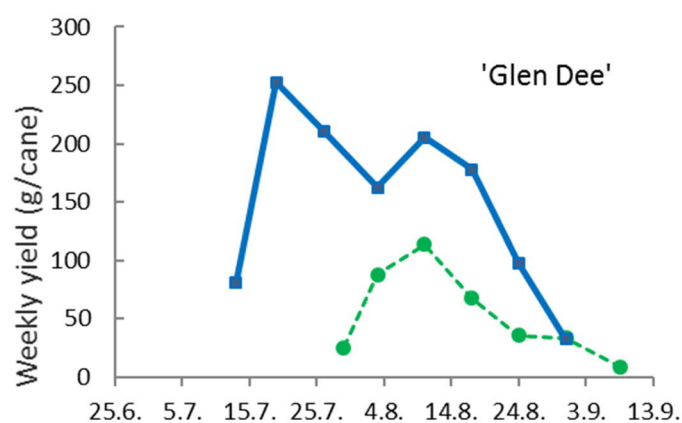
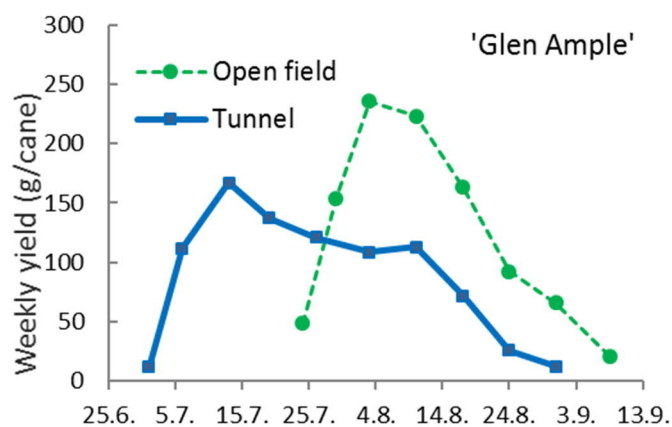
201

202 Table 1. Total yield, number of berries and average berry weight in three raspberry cultivars grown in open  
 203 field and high tunnel environments, 2015. The values are means of three replicates of five to six plants  
 204 each and followed by  $\pm$  SE.

	Yield (g/cane)	Number of berries /cane	Berry weight (g)
Open field			
'Glen Ample'	1003 $\pm$ 86 a	171 $\pm$ 4 a	5.6 $\pm$ 0.4 a
'Glen Dee'	367 $\pm$ 15 b	59 $\pm$ 1 b	6.2 $\pm$ 0.3 a
'Maurin Makea'	272 $\pm$ 20 b	71 $\pm$ 5 ab	3.6 $\pm$ 0.1 b
P	< 0.001	< 0.001	0.001
Tunnel			
'Glen Ample'	905 $\pm$ 102 b	224 $\pm$ 41	4.6 $\pm$ 0.8
'Glen Dee'	1272 $\pm$ 68 a	203 $\pm$ 5	6.3 $\pm$ 0.2
'Maurin Makea'	1082 $\pm$ 72 ab	227 $\pm$ 17	4.6 $\pm$ 0.1
P	0.052	Ns.	Ns.

205 Ns. = not significant

206 Cultivar means followed by a different letter, for both growing conditions separately, are significantly  
 207 different at  $P < 0.05$  by Tukey's test.

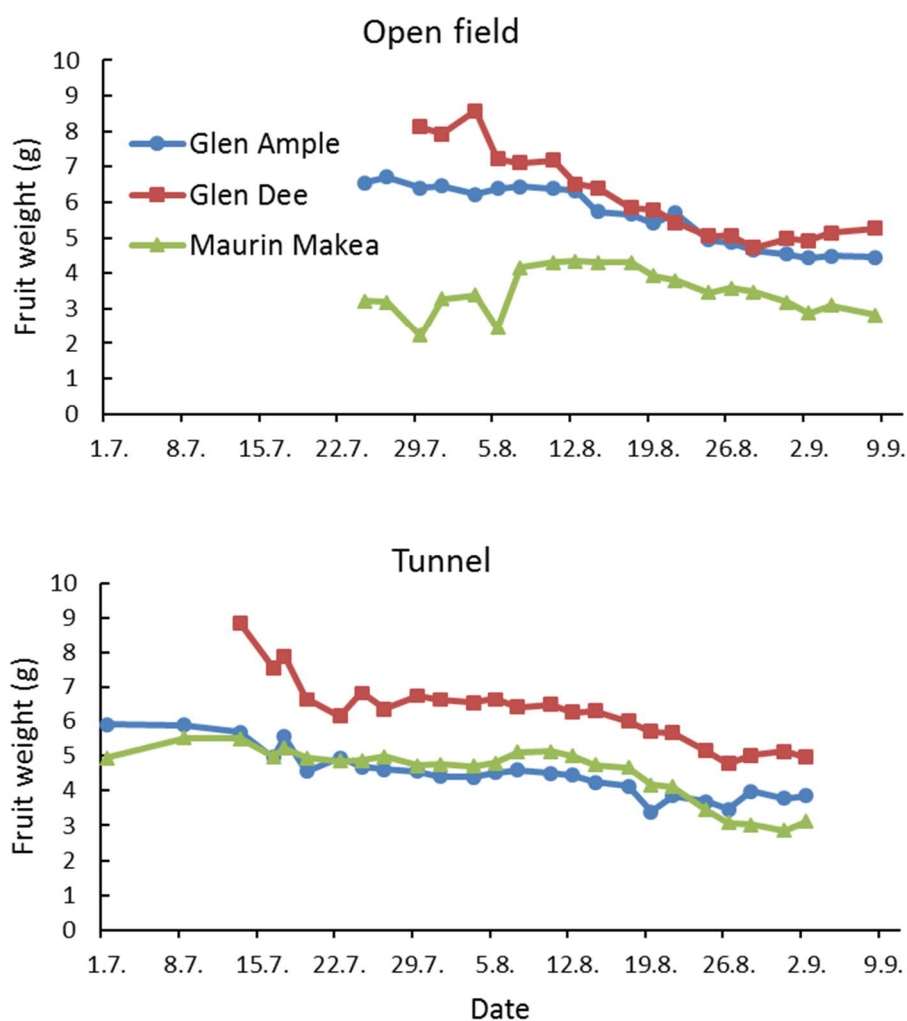


Date

208

209 Figure 3. Weekly marketable fruit yield per cane in three raspberry cultivars grown in open field and high  
 210 tunnel environments, 2015. The values are means of three replicates of five to six plants each.

211



212

213 Figure 4. Average berry weight during the harvest season in three raspberry cultivars grown in open field  
 214 and high tunnel environments, 2015. The values are means of three replicates of five to six plants each.

215

### 216 3.2. Berry quality

217 Berry sugar content and acidity were significantly affected by the growing conditions (Table 2). A higher  
 218 content of SS (°Brix) and TA were observed in berries from the open field, while the pH was lower. There  
 219 was no difference, however, in the sugar:acid ratio between the two growing conditions. Between the  
 220 cultivars, there were also differences; sugar content was lowest in 'Glen Dee' and the pH was lowest in  
 221 'Glen Ample' (Table 2).

222 In the open field, both pH ( $P = 0.002$ ) and TA ( $P = 0.012$ ) were significantly different between the cultivars,  
 223 with a pH of 3.00 for 'Glen Ample', 3.05 for 'Maurin Makea' and 3.07 for 'Glen Dee'. 'Glen Dee' berries were  
 224 also the least acidic based on the measurement of TA (Fig. 5). In the open field, the sugar content was  
 225 highest for 'Maurin Makea' and lowest for 'Glen Dee', but, according to the Kruskal-Wallis test, the  
 226 difference was not significant ( $P = 0.051$ ). In the tunnel, the differences between the cultivars in sugar  
 227 content, TA and pH were less pronounced and not significantly different.

228

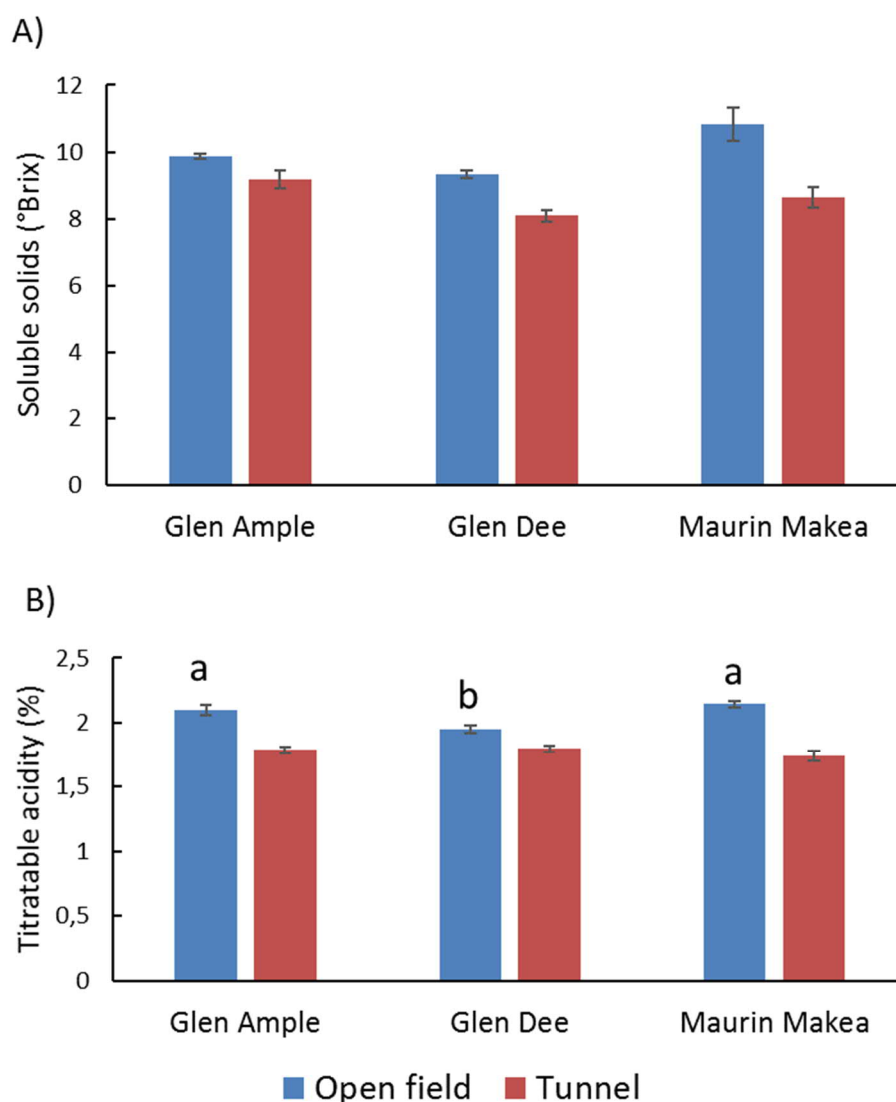
229 Table 2. The influence of growing conditions (open field or high tunnel) and cultivar on the concentration of  
 230 soluble solids ( $^{\circ}$ Brix) and titratable acids (TA), sugar:acid ratio, and pH in raspberry fruit juice. The values  
 231 are means of three replicates of five to six plants each and four sampling times, followed by  $\pm$  SE.

	$^{\circ}$ Brix	TA (g/100 g FW)	Sugar:acid ratio	pH
Growing condition (G)				
Open field	$10.0 \pm 0.3$	$2.06 \pm 0.03$	$4.86 \pm 0.11$	$3.04 \pm 0.01$
Tunnel	$8.6 \pm 0.2$	$1.77 \pm 0.02$	$4.88 \pm 0.12$	$3.26 \pm 0.01$
P	$< 0.001$	$< 0.001$	Ns.	$< 0.001$
Cultivar (C)				
'Glen Ample'	$9.5 \pm 0.2$ a	$1.94 \pm 0.07$	$4.93 \pm 0.15$	$3.12 \pm 0.05$ a
'Glen Dee'	$8.7 \pm 0.3$ b	$1.87 \pm 0.04$	$4.66 \pm 0.09$	$3.17 \pm 0.04$ b
'Maurin Makea'	$9.7 \pm 0.6$ a	$1.94 \pm 0.09$	$5.02 \pm 0.14$	$3.17 \pm 0.05$ b
P	0.008	Ns.	Ns.	0.001
Interaction				
G $\times$ C	Ns.	0.006	Ns.	Ns.

232 Ns. = not significant

233 Cultivar means followed by a different letter are significantly different at  $P < 0.05$  by Tukey's test.

234



235

236 Figure 5. Concentrations of A) soluble solids (°Brix) and B) titratable acids (%) in the fruit juice of three  
 237 raspberry cultivars grown in open field and high tunnel environments, 2015. The values are means of three  
 238 replicates of five to six plants each and four sampling times. Vertical bars present  $\pm$  SE. Cultivar means  
 239 marked by a different letter, for both growing conditions separately, are significantly different at  $P < 0.05$   
 240 by Tukey's test.

241

242 Examined all together, antioxidant activity was not affected by growing conditions or cultivar (Table 3).

243 When analysing data from the two growing conditions separately, however, cultivar differences were



244 observed in the open field ( $P = 0.042$ ), with antioxidant activity being higher for 'Glen Ample' berries than  
 245 'Glen Dee' (Fig. 6).

246 The TP content was not affected by growing conditions, but was affected by cultivar, with 'Glen Ample'  
 247 being significantly higher than 'Glen Dee' (Table 3). Furthermore, when the data from the two growing  
 248 conditions were analysed separately, this cultivar effect was still found to be significant in the open field ( $P$   
 249  $= 0.018$ ), where 'Glen Dee' berries had the lowest concentration of TP (Fig. 6). Additionally, a correlation  
 250 between antioxidant activity and the concentration of TP was also observed ( $r = 0.59$ ,  $P < 0.001$ ).

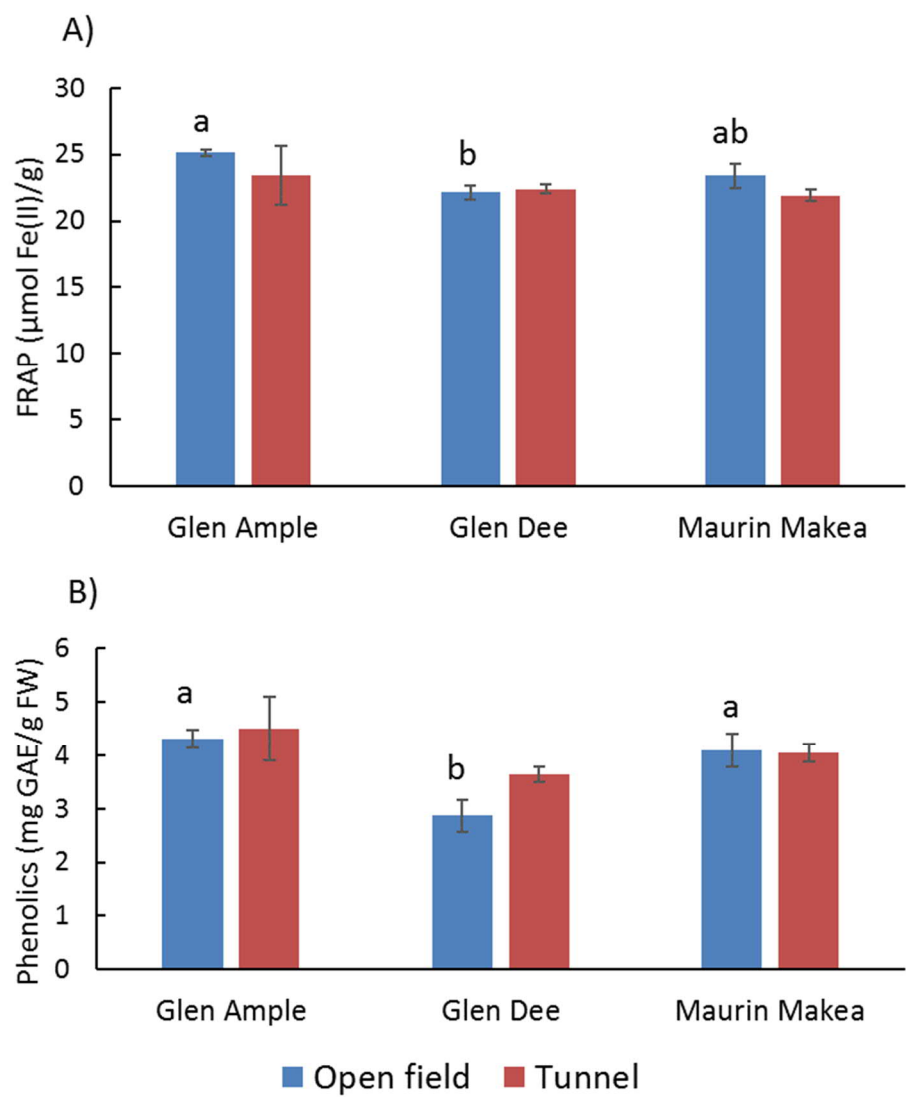
251

252 Table 3. The influence of growing conditions (open field or high tunnel) and cultivar on the antioxidant  
 253 activity (FRAP) and concentration of total phenolics in raspberry fruit in 2015. The values are means of  
 254 three replicates of five to six plants each and three sampling times and followed by  $\pm$  SE.

	FRAP ( $\mu\text{mol Fe(II)}/\text{g FW}$ )	Total phenolics (mg GAE/g FW)
Growing condition (G)		
Open field	$23.6 \pm 0.5$	$3.8 \pm 0.3$
Tunnel	$22.6 \pm 0.7$	$4.1 \pm 0.2$
P	Ns.	Ns.
Cultivar (C)		
'Glen Ample'	$24.3 \pm 1.1$	$4.4 \pm 0.3$ a
'Glen Dee'	$22.3 \pm 0.3$	$3.3 \pm 0.2$ b
'Maurin Makea'	$22.7 \pm 0.6$	$4.1 \pm 0.2$ ab
P	Ns.	0.011
Interaction		
G $\times$ C	Ns.	Ns.

255 Ns. = not significant

256 Cultivar means followed by a different letter are significantly different at  $P < 0.05$  by Tukey's test.



258

259 Figure 6. The A) antioxidant activity (FRAP) and B) concentration of total phenolics in the fruit of three  
260 raspberry cultivars grown in open field and high tunnel environments, 2015. The values are means of three  
261 replicates of four plants each and three sampling times. Vertical bars present ± SE. Cultivar means marked  
262 by a different letter, for both growing conditions separately, are significantly different at P < 0.05 by  
263 Tukey's test.

264

265 4. Discussion

266 To our knowledge, this is the first study to report the effect of protected cultivation of raspberry in  
267 polyethylene tunnels on fruit quality in Northern (60°13'N) conditions, where light conditions, including  
268 photoperiod and light spectral composition, are notably very different from other raspberry production  
269 areas. Fruit yield per cane was doubled in the tunnel compared to the open field. This has also been noted  
270 by previous studies for tunnel-grown florican raspberry cultivars, with more than double higher yields  
271 seen by Hanson et al. (2011) in Michigan, USA, Fernandez and Perkins-Veazie (2013) in North Carolina, USA,  
272 and Xu et al. (2014) in Quebec, Canada. Cropping potential is largely determined by the conditions during  
273 the season preceding the harvest year. In our experiment, the plants were also grown in the same  
274 conditions the previous year. In an earlier study we observed that the number of flowers (cropping  
275 potential) in 'Maurin Makea' long cane plants increased by 28-40% in tunnel-grown canes compared to the  
276 field-grown canes, depending on the duration of cold storage (Palonen et al., 2015).

277 The temperatures inside the tunnel were relatively high, especially between late May and early July.  
278 Remberg et al. (2010) recommended relatively low temperatures, 12 to 18°C, for protected raspberry  
279 production since berry weight decreases with increasing post-flowering temperature. Although the  
280 temperatures recorded in our tunnel were much higher, the berry size was not reduced. In the study by  
281 Fernandez and Perkins-Veazie (2013), berry size was not affected by tunnel growing conditions either.  
282 Some of the 'Glen Ample' fruit in the tunnel were crumbly, a disorder that is increasingly common in  
283 Europe, and has recently been shown to depend on seasonal and environmental, as well as genetic, factors  
284 (Graham et al., 2015). The tunnel growing conditions may have influenced the expression of this condition  
285 in our experiment.

286 Berries grown in the open field had higher contents of SS (°Brix) and higher TA than those grown in the high  
287 tunnel, whereas the sugar:acid ratio was not affected. Furthermore, the differences between the cultivars  
288 regarding these attributes were more pronounced in the open field. Sugar content and acidity only partially  
289 explain sensory taste, with 15-20 volatile aroma compounds detected in raspberry fruit significantly  
290 affecting its flavor (Larsen and Poll, 1990). Sensory sweetness and sensory acidity do correlate with

291 measured sucrose content and sugar:acid ratio (Stavang et al., 2015), although the sugar:acid ratio may be  
 292 more closely related to fruit maturity.

293 Warm and dry weather increases sugar content and decreases acidity in raspberry fruit (Jennings, 1988).  
 294 Malowicki et al. (2008) observed that, for raspberries grown in Oregon and Washington, the SS were higher  
 295 and TA lower for the growing site with higher average temperatures. In our study, SS was, on average,  
 296 10.0% in the open field and 8.6% in the high tunnel, and was lowest for 'Glen Dee'. Lower SS in the tunnel  
 297 compared to the open field has also been reported for the primocane-type raspberry 'Polka' in Poland  
 298 (Król-Dyrek and Siwek, 2015). Norwegian researchers have reported SS contents of 8.3% for 'Glen Ample' in  
 299 the open field (61°11'N) (Mazur et al., 2014a) and, in another study, 9.5% in a polyethylene tunnel  
 300 (59°40'N) (Remberg et al., 2010). The SS for 'Glen Ample' in our study was 9.9% in the open field and 9.2%  
 301 in the tunnel. The TA measured in our study for 'Glen Ample' in the open field was exactly the same (2.1  
 302 g/100 g FW) as observed in the open field in Norway (Mazur et al., 2014a). For cultivars 'Glen Clova' and  
 303 'Glen Prosen', TA concentrations of 1.3% and 2.0%, respectively, have been reported; the average for 40  
 304 red raspberry genotypes being 1.6% (Weber et al., 2008)

305 Raspberry plant photosynthesis declines as temperatures rise above 25°C (Stafne et al., 2001). It is possible  
 306 that high temperatures inside the tunnel led to decreased transport of photosynthates into the fruit.

307 Environmental conditions including temperature, light and humidity also affect the biosynthesis of flavor  
 308 volatiles (Paterson et al., 2013). We observed that for some cultivars flavor did not develop as well in the  
 309 tunnel as in the open field.

310 Fruit bioactive properties, including phenolic compounds and antioxidant activity, were not affected by the  
 311 high tunnel. In 'Glen Ample' fruit, TP concentrations of 1.9 mg GAE/g FW in the open field (Mazur et al.,  
 312 2014a) and 2.6 mg GAE/g FW in the tunnel (Remberg et al., 2010) have been reported. In these studies, the  
 313 Folin-Ciocalteu method was used. In 'Glen Ample', we obtained measures of, on average, 4.4 mg GAE/g FW  
 314 using the Fast Blue BB method which has been observed to yield a 2.6-fold increase in concentration of TP,  
 315 compared to the Folin-Ciocalteu method (Lester et al., 2012). TP concentrations for 'Maurin Makea' and

316 'Glen Dee' fruit have not been previously reported in the literature, though, for 12 red raspberry cultivars  
317 grown in Eastern Finland, the phenolic concentrations ranged from 1.92 to 3.59 mg/g FW (Folin-Ciocalteu)  
318 (Anttonen and Karjalainen, 2005). Weber et al. (2008) reported TP concentrations of 3.78 and 3.70 mg / g  
319 FW in cultivars 'Glen Clova' and 'Glen Prosen', respectively; the average for 40 red raspberry genotypes  
320 being 4.21 mg / g FW. Comparing results from different studies is further complicated by the fact that the  
321 recovery of phenolic compounds, and thus their measured concentration, is dependent on the extracting  
322 solvent used (Addai et al., 2013).

323 Many environmental factors influence the biosynthesis of phenolic compounds in plants, and the regulation  
324 appears to be complex. High temperatures, for example, inhibit anthocyanin production in apple (Lin-Wang  
325 et al., 2011). On the other hand, in strawberry, high temperature (30/22°C day/night) increased the content  
326 of phenolic compounds and antioxidant capacity (Wang and Zheng, 2001). Additionally, an interaction  
327 between the genotype and environmental conditions on the antioxidant activity in raspberry was observed  
328 by Hanson et al. (2011). In our study as well, high temperatures inside the tunnel may have changed the  
329 fruit's chemical composition and relative amounts of individual phenolic compounds, although the total  
330 concentration of phenolics was not affected. Cultivar differences were pronounced, with 'Glen Ample' fruit  
331 having the highest concentration of TP and 'Glen Dee' the lowest. A direct relationship between the  
332 antioxidant capacity and the content of TP, especially anthocyanins and ellagitannins, has been shown for  
333 raspberry fruit (Wang and Lin, 2000; Liu et al., 2002; Remberg et al., 2010). A correlation was observed in  
334 our study as well. Antioxidant activity showed a trend similar to TP, although the differences between the  
335 cultivars were not as pronounced. Weber et al. (2008) reported FRAP values of 21 and 20 Trolox  
336 equivalents in cultivars 'Glen Clova' and 'Glen Prosen', respectively; the average for 40 red raspberry  
337 genotypes being 25. The average in our study was 23.1  $\mu\text{mol Fe(II)}/\text{g}$ , across all cultivars and both growing  
338 conditions.

339 The data presented here can not be used to determine a single environmental factor that caused the  
340 differences in berry quality, but rather to compare two different production systems and environments.

341 Not only were temperature and light conditions, humidity and windiness different, but the plants were  
342 managed differently in each growing condition. Key production practices, such as irrigation, fertilization,  
343 and growth substrate were different. Furthermore, harvest maturity affects all these measured parameters.  
344 For example, TA decreases and the concentration of anthocyanins and the sugar:acid ratio (mainly due to  
345 decreasing acidity) increase during raspberry fruit maturation (Stavang et al., 2015). Therefore, it is crucial  
346 for the reliability of any analysis that sampled berries are in the same maturity range.

347 While the content and composition of phenolic compounds are certainly affected by environmental  
348 conditions, it appears that these characteristics are more greatly affected by genotype. Anttonen and  
349 Karjalainen (2005) reported large variation in fruit TP concentration between the raspberry cultivars grown  
350 in Finland, ranging from 192 mg/100 g (FW) in 'Gatineau' to 359 mg/100 g (FW) in 'Ville'. Differences were  
351 also observed in the contents of quercetin, ellagic acid, and anthocyanins. In our study, the open field TP  
352 concentration of 'Glen Ample' was 48% higher than that of 'Glen Dee'. The highest contents of phenolics  
353 have actually been measured in wild raspberry (Määttä-Riihinen et al., 2004). Weber et al. (2008) reported  
354 a threefold difference in FRAP values across red raspberry genotypes. Apparently, it is more important to  
355 consider the genotype grown than the influence of growing conditions, as genotype effects are far more  
356 significant.

357 In conclusion, raspberry production under polyethylene tunnels may provide major benefits, as fruit yield  
358 per cane was doubled in the tunnel compared to the open field, while fruit bioactive properties, including  
359 phenolic compounds and antioxidant activity, were not affected. Berries grown in the open field, however,  
360 contained more sugars and acids than the ones grown in the tunnel. Cultivar differences were pronounced,  
361 with 'Glen Ample' and 'Maurin Makea' berries having higher phenolic concentrations and being sweeter  
362 than 'Glen Dee' berries.

363

364 Acknowledgements

365 We gratefully acknowledge financial support for this work from the Ministry of Agriculture and Forestry,  
366 Finland (Grant no. 1900/312/2013) and from the Maiju and Yrjö Rikala Horticultural Foundation. We also  
367 thank the technical staff at the research greenhouses and laboratories of the University of Helsinki.  
368 Professor Salla Karhu is acknowledged for her help with the statistical analysis.

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